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COMPARATIVE MORPHOLOGICAL ANALYSIS OF TESTIS FOLLICLES IN DUNG BEETLES (COLEOPTERA: SCARABAEIDAE: SCARABAEINAE, APHODIINAE, GEOTRUPINAE)

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Abstract.—Testis follicles and sperm size of various Scarabaeinae, Aphodiinae, and Geotrupinae species were compared. Geotrupinae and Scarabaeinae species have 6 follicles per testis, except *Digitonthophagus gazella,* which has 12. In Aphodiinae, depending on tribe, some species have 7 follicles per testis (Aphodiini), with all follicles of the same size or with 2 large and 5 small follicles; other Aphodiinae (Eupariini) have only 2 follicles per testis. In Scarabaeinae and Aphodiinae, the follicles are not septate, while in Geotrupinae they are. Scarabaeinae spermatozoa measure from 90 to 600 μm, Geotrupinae spermatozoa from 116 a 166 μm. Aphodiinae spermatozoa length varies notably from tribe to tribe—in Aphodiini, from 500 to 2,000 μm, in Eupariini from only 110 to180 μm.

Resumen.—En varias especies de Scarabaeinae, Aphodiinae y Geotrupinae se estudiaron comparativamente los folículos testiculares y los espermatozoides. En Geotrupinae y Scarabaeinae hay seis folículos por testículo excepto en *Digitonthophagus gazella* que tiene doce. Mientras que en Aphodiinae, según la tribu, puede haber siete folículos por testículo del mismo tamaño o de dos tamaños: dos grandes y cinco pequeños (Aphodiini) o sólo dos folículos por testículo (Eupariini). En Scarabaeinae y Aphodiinae los folículos no son septados, mientras que en Geotrupinae son septados. Los espermatozoides en Scarabaeinae miden de 90 a 600 μm. En Geotrupinae miden de 116 a 166 μm. En Aphodiinae la longitud de los espermatozoides es muy diferente de una tribu a otra, en Aphodiini los espermatozoides miden de 500 a 2,000 μm y en Eupariini sólo de 110 a 180 μm.

Key Words: dung beetles, testis follicles, spermatozoa, escarabajos coprófagos, folículo testicular, espermatozoides

Most dung beetles of the subfamilies Scarabaeinae, Aphodiinae, and Geotrupinae depend on the excrement of herbivores, owing to these beetles' feeding and reproductive behaviors. Dung is used for nesting and depositing eggs, and as food for both developing and adult individuals. For these reasons, dung beetles play a critical role in maintaining grasslands: in using dung, they reduce its presence on the ground, prevent the loss of elements like nitrogen that the dung contains, and increase soil fertility (Halffter and Mathews 1966, Halffter and Edmonds 1982, Rougon et al. 1988, Hanski and Cambefort 1991).

The anatomy and morphology of male genitalia of many species in these subfamilies have been studied for taxonomic purposes (Sharp and Muir 1912, Zunino 1983, 1984, D'Hotman and Scholtz 1990). Nevertheless, few aspects of the internal structure of testis follicles are known for some Scarabaeinae species (Virkki 1956, 1957, Benítez and Martínez 1985, Martínez and Benítez 1988, Martínez and Cruz 1992). There is also a lack of information on Aphodiinae (Virkki 1951, 1953, 1956, 1957) and Geotrupinae (Virkki 1951, 1956, 1957, López-Guerrero and Benítez 1982, López-Guerrero 1987). Nothing about the spermatozoa of any of these species has been studied previously.

Given the biological and ecological importance of these subfamilies and the lack of knowledge about male reproductive anatomy among their species, the present investigation made a comparative analysis of the microscopic structure of testis follicles and spermatozoa size in various species of Scarabaeinae, Aphodiinae, and Geotrupinae.

Materials and Methods

A number of dung beetle species were studied, representing the principal tribes of the three subfamilies. Most of these species were collected in Mexico, some in France and Uruguay. The species studied were as follows:

Scarabaeinae (*sensu* Halffter 1997): Onthophagini: *Onthophagus batesi* Howden and Cartwright, *O. chevrolati* Harold, *O. incensus* Say, *O. cyanellus* Bates, *O. rhinolophus* Harold, *O. hippopotamus* Harold, *O. aureofuscus* Bates, and *Digitonthophagus gazella* (Fabricius). Onitini, Gromphina: *Gromphas lacordairei* (Brullé). Phanaeina: *Phanaeus tridens* Laporte, *P. amethystinus* Harold, *P. endymion* Harold, *P. damon* Castelnau, *Coprophanaeus telamon* (Harold), *C. pluto* (Harold), and *Sulcophanaeus menelas* Laporte. Oniticellini, Oniticellina: *Euoniticellus intermedius* (Reiche) and *Liatongus rhinocerulus* (Bates). Coprini, Coprina: *Copris laeviceps* Harold, *C. incertus* Say, *C. lugubris* Boheman, *C. armatus* Harold, and *C. klugi sierrensis* Matthews. Dichotomiina: *Dichotomius satanas* (Harold), *D. carolinus* (Say), *D. centralis* (Harold), *Canthidium moestum* Harold, *Ateuchus illaesum* Harold, and *Ontherus mexicanus* Harold. Eurysternini: *Eurysternus mexicanus* Harold and *E. caribaeus* (Herbst). Scarabaeini, Canthonina: *Canthon indigaceus chevrolati* Harold, *C. i. chiapas* Robinson, *C. cyanellus cyanellus* LeConte, *C. viridis viridis* Martínez, Halffter, and Halffter, *C. humectus* (Say), *C. (Glaphyrocanthon) femoralis femoralis* Chevrolat, *C. (G.) viridis leechi* Martínez, Halffter and Halffter, *C.(G.) subhyalinus* Harold, *Deltochilum pseudoparile* Paulian, *D. lobipes* Bates, and *D. gibbosum* (Fabricius).

Aphodiinae (*sensu* Endrödi 1966): Aphodiini: *Aphodius (Teuchestes) fossor* (L.), *A. (Colobopterus) erraticus* (L.), *A. (Calamosternus) granarius* (L.), *A. (Labarrus) pseudolividus* Balthasar, *A. (Trichaphodius) opisthius* Bates, *A. (Platyderides) fuliginosus* Harold, and *A. (Bodilus) sallei* Harold. Eupariini: *Ataenius cribrithorax* Bates, *A. sculptor* Harold, *A. sp. aff. perforatus* Harold, *A. sp. aff. cognatus* LeConte.

Geotrupinae (*sensu* Zunino 1984): Geotrupini: *Megatrupes cavicollis* Bates, *Anoplotrupes stercorosus* (Scriba), and *Trypocopris vernalis* (L.). Ceratotrupini: *Halffterius rufoclavatus* (Jekel), *Ceratotrupes bolivari* Halffter and Martínez, *Onthotrupes herbeus* (Jekel), *O. sobrinus* (Jekel), and *O. nebularum* (Howden).

For each species, 5 to 10 males were dissected in Ringer solution, and their reproductive apparatus fixed in AFATD (ethyl alcohol 96°–formaldehyde–trichloroacetic acid–dimethylsulfoxide) (Carayon 1969). To study the structure of the testicular follicles, most were stained with Feulgen-green light and mounted in their entirety. Other follicles were imbedded in Histosec®, and histological sections of 7 μm were stained with PAS-Hematoxylin (Gabe 1968). To study spermatozoa, a follicle smear was taken from mature males, fixed in methanol, and stained with Giemsa (Clark 1973).

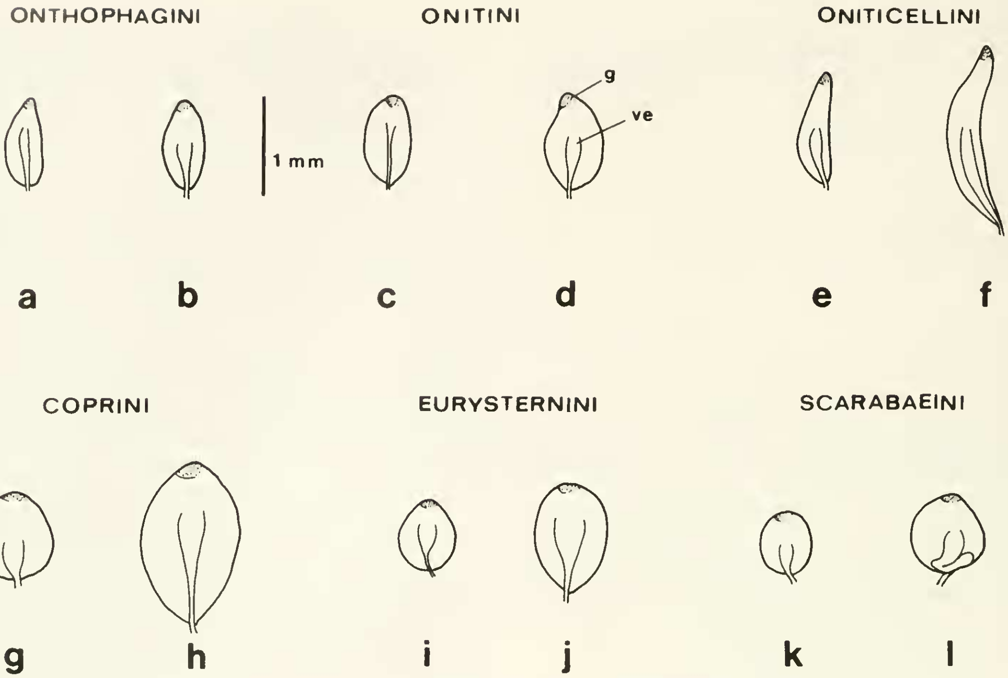

Fig. 1. Schematics of testis follicle forms in species of the principal Scarabaeinae tribes. Onthophagini: a, *Onthophagus batesi*; b, *Digitonthophagus gazella*. Onitini: c, *Gromphas lacordairei*; d, *Phanaeus tridens*. Oniticellini: e, *Euoniticellus intermedius*; f, *Liatongus rhinocerulus*. Coprini: g, *Copris incertus*; h, *Dichotomius satanas*. Eurysternini: i, *Eurysternus mexicanus;* j, *Eurysternus caribaeus*. Scarabaeini: k, *Canthon indigaceus chevrolati*; l, *Deltochilum pseudoparile*. (g = germarium; ve = vas efferens).

Results

Scarabaeinae

Males of the Scarabaeinae have two testes, each with six follicles, with the exception of *D. gazella,* which has 12 follicles per testis (Pluot-Sigwalt and Martínez 1998). The form of the follicles is variable—they can be spherical, ovoids, or very elongated ovoids (Fig. 1)—and their coloration *in vivo* varies with species, independently of sexual maturity.

In Onthophagini (Figs. 1a, b), follicles can be very elongated ovoids, either white (*Onthophagus batesi, O. incensus, O. cyanellus, O. rhinolophus*) or yellowish (*O. chevrolati, O. hippopotamus, D. gazella*). In Onitini (Figs. 1c, d), they are ovoids, yellowish (*Phanaeus tridens, P. demon, P. amethystinus, Coprophanaues pluto, Gromphas lacordairei*) or orangish (*P. endymion, C. telamon, Sulcophanaeus menelas*). In Oniticellini (Figs. 1e, f), they can be very elongated white ovoids (*Euoniticellus intermedius, Liatongus rhinocerulus*). Coprini Coprina (Fig. 1g) follicles can be spherical, whether white (*Copris incertus, C. armatus, C. klugi sierrensis*), yellowish (*C. laeviceps*), or orangish (*C. lugubris*); while in Dichotomiina (Fig. 1h), follicles are ovoids, whether orangish (*Dichotomius satanas, D. carolinus, D. centralis*), yellowish (*Canthidium moestum*), or white (*Ateuchus illaesum, Ontherus mexicanus*). Eurysternini (Figs. 1i, j) present elongated ovoids that are white (*Eurysternus mexicanus*) or yellow (*E. caribaeus*). Scarabaeini (Figs. 1k, l) follicles are spherical, either white (*Canthon cyanellus cyanellus, C. viridis viridis, C. humectus*), yellowish (*C. [Glaphyrocanthon] femoralis femoralis, C. [G.] viridis leechi, C. [G.] subhyalinus, Deltochilum gibbosum*), or orangish (*Canthon indiga-*

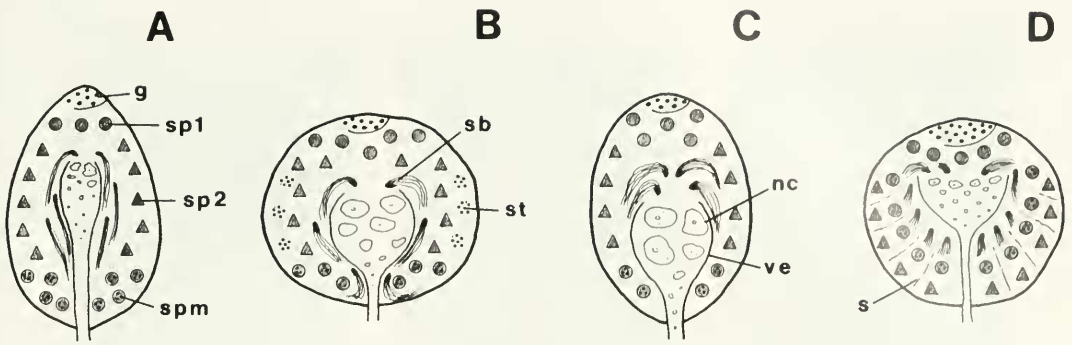

Fig. 2. General scheme of testis follicle structure in A, Scarabaeinae; B, Aphodiini; C, Eupariini; D, Geotrupinae. (g = germarium; nc = nurse cells; s = septa; sb = sperm bundles; sp1 = primary spermatocytes; sp2 = secondary spermatocytes; spm = spermatids; st = spermatozoa tails, transverse sections; ve = vas efferens).

ceus chevrolati, C. i.chiapas, D. pseudoparile, D. lobipes). The follicles of Dichotomiina and Eurysternini are notable for their strong similarity to each other.

In all the Scarabaeinae species studied, each follicle shows radial symmetry and is externally defined by a cellular wall. In the apical region, the germarium is separated from other follicular tissue by an acellular membrane; in the central region, the vas efferens is found, and around the vas efferens, cysts in different spermatogenesis stages (Figs. 2A, 3A).

The cellular wall that defines each follicle varies in thickness according to the species, with the thickest walls seen in *C. telamon, D. satanas, E. caribaeus,* and *D. gibbosum.* In all Scarabaeinae species, the germarium contains abundant spermatogonia. The vas efferens begins relatively close to the germarium and is straight, taking the form of a funnel, with the anterior region open and more enlarged than the posterior region. Inside the vas efferens, in mature males, are abundant nurse cells and sperm bundles. Upon leaving the follicles, the vas efferens narrows until it joins the vas deferens. Depending on tribe, the anterior part of the vas efferens can be more or less enlarged, while the posterior region is straight, except in the genus *Deltochilum,* in which it is bent toward the base of the follicle (Fig. 1,l). The nurse cells in the vas efferens of mature males measure 22 to 66 μm in diameter, depending on species (Figs. 3A, B, C).

The arrangement of cysts in different maturational stages is constant within any tribe. Cysts of spermatogonia and primary spermatocytes are located under the germarium. Secondary spermatocyte cysts migrate toward the periphery, from the anterior to the posterior region of the follicle. Spermatid cysts are found in the posterior and central region of the follicle and toward the posterior part of the vas efferens. Maturing sperm adhere to nurse cells and are found around the vas efferens from the posterior to the anterior region. The nurse cells have a strong PAS+ reaction.

Sperm bundles are generally found parallel to the direction of the vas efferens in long ovoid follicles, or in an irregular pattern of spherical or ovoid follicles. They occur abundantly at the entrance of the vas efferens and within it, where they are found free among degenerating nurse cells (Figs. 2A, 3A, B, C). Within the sperm bundles of most species, spermatozoa tails are elongated and do not undulate much, but they can also be found balled up, as in *C. telamon, G. lacordairei, D. satanas, C. moestum,* and *A. illaesum,* or in a zig-zag form, as in *P. tridens* and *D. carolinus.*

Spermatozoa are tailed in all species of this subfamily. Cell length varies from 90 to 600 μm, depending on tribe. In Onthophagini species, spermatozoa length is 320

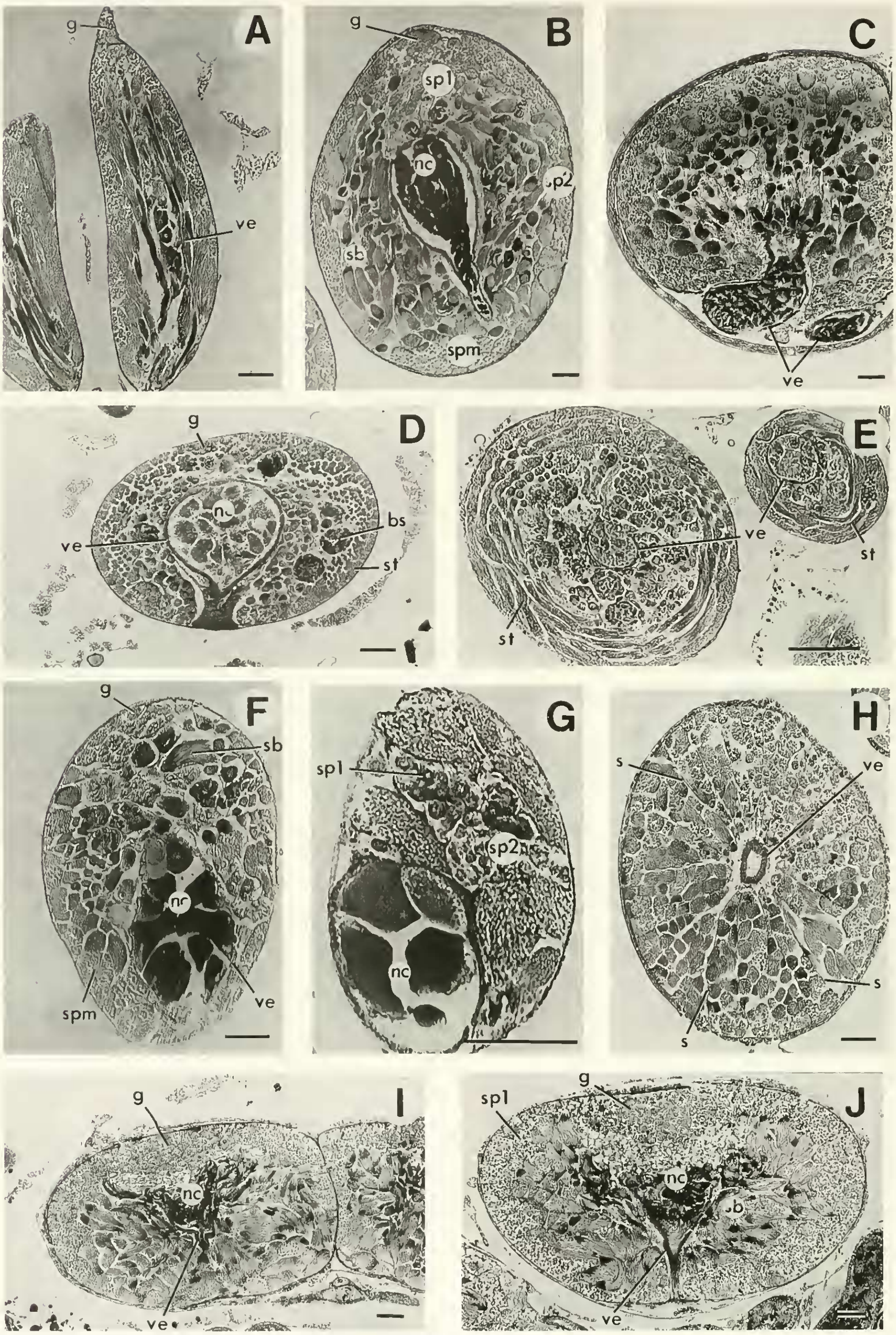

Fig. 3. Microphotographs of histological sections of the testis follicles of species in the three subfamilies. Scarabaeinae: longitudinal sections in A, *Onthophagus batesi*; B, *Eurysternus caribaeus* and C, *Deltochilum gibbosum*. Aphodiinae, Aphodiini: D, *Aphodius fossor* in longitudinal section and E, *Aphodius sallei*, follicles of two different sizes in transverse section. Eupariini: longitudinal sections in F, *Ataenius sculptor* and G, *Ataenius cribrithorax*. Geotrupinae: H, *Anoplotrupes stercorosus* in transverse section and longitudinal sections

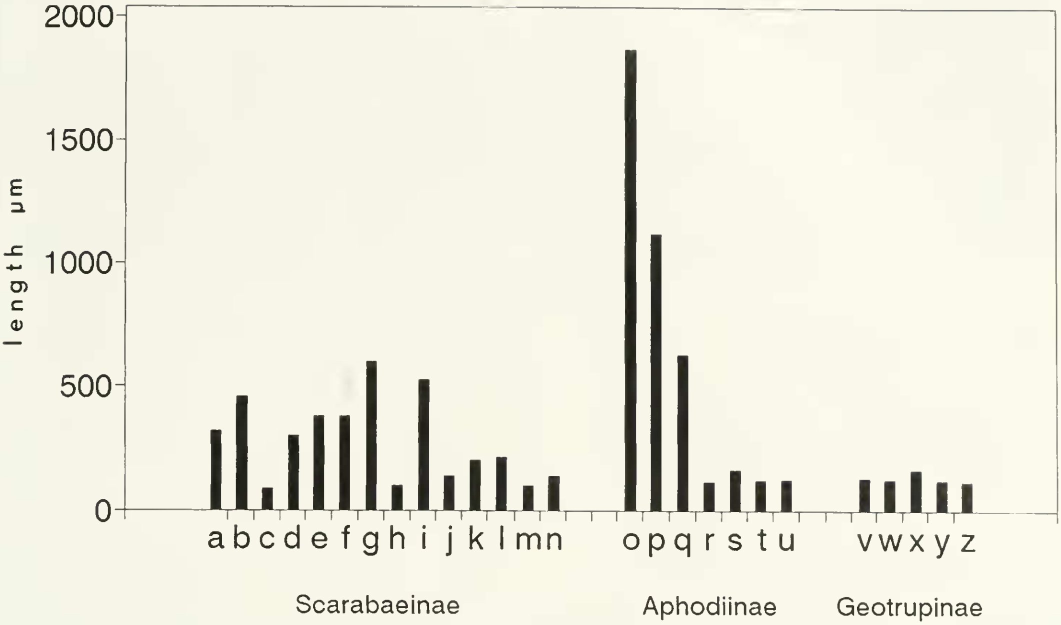

Fig. 4. Graph illustrating spermatozoa size in species of the three subfamilies. Scarabaeinae: a, *Onthophagus batesi*; b, *Onthophagus cyanellus*; c, *Digitonthophagus gazella*; d, *Phanaeus amethystinus*; e, *Sulcophanaeus menelas*; f, *Euoniticellus intermedius*; g, *Liatongus rhinocerulus*; h, *Copris laeviceps*; i, *Dichotomius carolinus*; j, *Ontherus mexicanus*; k, *Eurysternus mexicanus*; l, *Eurysternus caribaeus*; m, *Canthon cyanellus cyanellus*; n, *Deltochilum pseudoparile*. Aphodiinae: o, *Aphodius opisthius*; p, *Aphodius sallei*, spermatozoa from the large follicle; q, *Aphodius sallei*, spermatozoa from the small follicle; r, *Ataenius cribrithorax*; s, *Ataenius sculptor*; t, *Ataenius sp. aff. perforatus*; u, *Ataenius sp. aff. cognatus*. Geotrupinae: v, *Anoplotrupes stercorosus*; w, *Megatrupes cavicollis*; x, *Ceratotrupes bolivari*; y, *Onthotrupes nebularum*; z, *Halffterius rufoclavatus*.

to 460 µm, except in *D. gazella*, whose much smaller spermatozoa measure about 90 µm (Figs. 4a, b, c). In Onitini, spermatozoa length is 300 to 380 µm. The shortest spermatozoa are found in *P. amethystinus* and *C. pluto*, the longest in *S. menelas* (Figs. 4d, e). Among Oniticellini species, the spermatozoa length of *E. intermedius* is about 380 µm, that of *L. rhinocerulus* 600 µm (Figs. 4f, g). In Coprini Coprina, spermatozoa measure between 100 and 120 µm (Fig. 4h). For Dichotomiina, spermatozoa length is from 320 to 530 µm (as in *D. carolinus*), except in *O. mexicanus*, which has a spermatozoa length of 140 µm (Figs. 4i ,j). In the two Eurysternini species, spermatozoa length is about 200 µm (Figs. 4k, l). Scarabaeini Canthonina species have spermatozoa lengths from 100 to 160 µm (Figs. 4m, n).

Aphodiinae

In Aphodiinae, the number of testes follicles varies with tribe (Pluot-Sigwalt and Martínez 1998). Males of the Aphodiini tribe have seven spherical white follicles in each of two testes. In some of these species, all seven follicles are of the same size (*A. fossor, A. opisthius*); in others, there are two large follicles and five smaller follicles,

←

in I, *Onthotrupes sobrinus* and J, *Onthotrupes nebularum*. (bs = balled sperm bundles; g = germarium; nc = nurse cells; s = septa; sb = sperm bundles; sp1 = primary spermatocytes; sp2 = secondary spermatocytes; spm = spermatids; st = spermatozoa tails; ve = vas efferens). The scale represents 100µm.

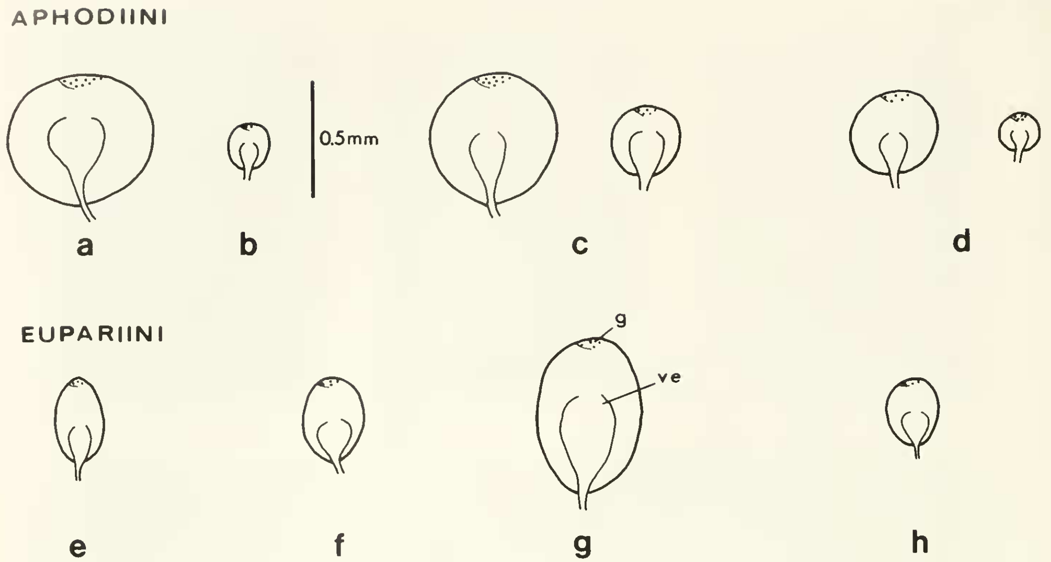

Fig. 5. Schemes showing the form of testis follicles in the two Aphodiinae tribes. Aphodiini with follicles of the same size: a, *Aphodius fossor*; b, *Aphodius opisthius*. Aphodiinae with two sizes of follicle: c, *Aphodius fuliginosus*; d, *Aphodius pseudolividus*. Eupariini: e, *Ataenius cribrithorax*; f, *Ataenius sp. aff. perforatus*; g, *Ataenius sculptor*; h, *Ataenius sp. aff. cognatus*. (g = germarium; ve = vas efferens).

with the smaller follicles measuring about half the size of the larger ones (*A. erraticus, A. granarius, A. lividus, A. fuliginosus, A. sallei*). In contrast, Eupariini species have just two white, ovoid follicles of the same size in each testis (Fig. 5).

In Aphodiini species, each follicle, independent of its size, has an external cellular wall, a germarium in the apical region that is basally bounded by a membrane, and a central vas efferens with cysts in various states of development around it (Figs. 2B, C). The germarium contains few spermatogonia. The vas efferens takes the form of a capsule, which is very enlarged in the anterior region, and in mature males, contains large, degenerating nurse cells (Figs. 3D, F). All follicles, whether large or small, show a paucity of cysts in different stages of spermatogenesis, but these cysts are large. Follicles of both sizes are functional. Cyst distribution is roughly similar to that described for Scarabaeinae species. Beneath the germarium, toward the entrance of the vas efferens, are primary spermatocyte cysts that are large but few in number. Secondary spermatocyte cysts are found in the middle region of the follicle toward the periphery and in the posterior region. Spermatid cysts are present in the posterior region around the vas efferens. Young sperm bundles have spermatozoa with short tails, take the form of balls, and are grouped around the anterior part of the vas efferens. The most notable features of the Aphodiini species studied are the distribution of mature sperm bundles and spermatozoa size. Sperm bundles are located around the vas efferens, but spermatozoa tails are so long that they curl up several times before meeting the periphery, and thus are found under the follicle wall from the apical to the basal region of the follicle. In a longitudinal section of the follicle, the spermatozoa tails under the follicle wall are therefore seen cut transversely (Figs. 2B, 3D, E). Nurse cells found in the vas efferens measure from 17 to 50 μm in diameter (Figs. 3D, E).

Aphodius opisthius follicles are about the same size, measuring about 215 μm in diameter, and their spermatozoa measure approximately 2,000 μm. Species with follicles of two different sizes, such as *A. sallei,* with 2 follicles measuring about 300 μm in diameter and 5 follicles measuring about 150 μm in diameter, have spermatozoa of two different sizes. In these species, large-follicle spermatozoa are about 1,000 μm in length, while small-follicle spermatozoa measure about 500 μm (Figs. 4o, p, q).

Testis follicles of Eupariini species follow the same general scheme as in Aphodiini (Fig. 2C). These species also have large but few cysts in various maturational stages. Sperm bundles are found around the entrance of the vas efferens, but they are not beneath the follicle wall. Balled sperm bundles are also observed, but in lesser quantity than in Aphodiini. The nurse cells found within the vas efferens are very large, from 49 to 93 μm in diameter (Fig. 3F). The spermatozoa measure only 110 to 200 μm in length (Figs. 4r, s, t, u).

Geotrupinae

Males of the Geotrupinae subfamily have two testes, with six follicles in each (Pluot-Sigwalt and Martínez 1998). The follicles are spherical and white in all the species studied (Fig. 6).

In species of the two Geotrupinae tribes, follicles have a thick external cellular wall, a relatively large germarium with abundant spermatogonia, and a vas efferens, with abundant cysts in different stages of development. In both tribes, testis follicles have radially organized septa, with the septa more notable in Geotrupini than in Ceratotrupini. The vas efferens begins very close to the germarium and has a conical form, with a very open anterior region, and a thick wall in the posterior region (Figs. 2D, 3G, H).

Because of the presence of septa, the distribution of cysts is very different from that observed in Scarabaeinae and Aphodiinae. Cysts of primary spermatocytes are found beneath the germarium and beneath the follicle wall. Found in each septa, toward the center of the follicle, are secondary spermatocyte cysts, spermatid cysts, and sperm bundles. Within the entrance of the vas efferens, abundant sperm bundles are found together with nurse cells. Within the vas efferens itself are numerous degenerating nurse cells, measuring 17 to 20 μm in diameter (Figs. 3G, H). Spermatozoa length does not vary much in Geotrupini and Ceratotrupini species—from 116 a 166 μm (Figs. 4v, w, x, y, z).

Comparison of the Subfamilies and Discussion

These observations of testis follicles and spermatozoa size in Scarabaeinae, Aphodiinae, and Geotrupinae show that both similarities and differences are found at the levels of subfamily, tribe, and genus.

Morphology of testis follicles.—The form of testis follicles varies according to subfamily, and in some subfamilies, according to tribe (Figs. 1, 5, 6). The greatest variation in tribes was seen in Scarabaeinae, whose follicles vary from elongated ovoids to spheres. The most elongated follicles of all were seen in Onthophagini and Oniticellini, the most spherical in Coprini and Canthonina. In Aphodiinae, ovoid follicles are seen in Eupariini, and spherical follicles in Aphodiini. In the two Geotrupinae tribes, follicles are spherical. According to Virkki (1957), long ovoid follicles are more primitive than spherical ones. From this perspective, in Scarabaeinae the most primitive follicle state would be those of Onthophagini and Oniticellini, and the most evolved in Coprini and Canthonina. In Aphodiinae, the most evolved follicles would be those of Aphodiini. In Geotrupinae, no differences are seen: both of the two tribes have spherical follicles.

Microscopic structure of testis follicles.—The microscopic structure of testis follicles is similar in Scarabaeinae and Aphodiinae, but quite different in Geotrupinae (Figs. 2, 3). The follicles are not septate in Scarabaeinae and Aphodiinae, while in Geotrupinae they are—differences that Virkki (1951, 1957) had already observed in some species among these subfamilies.

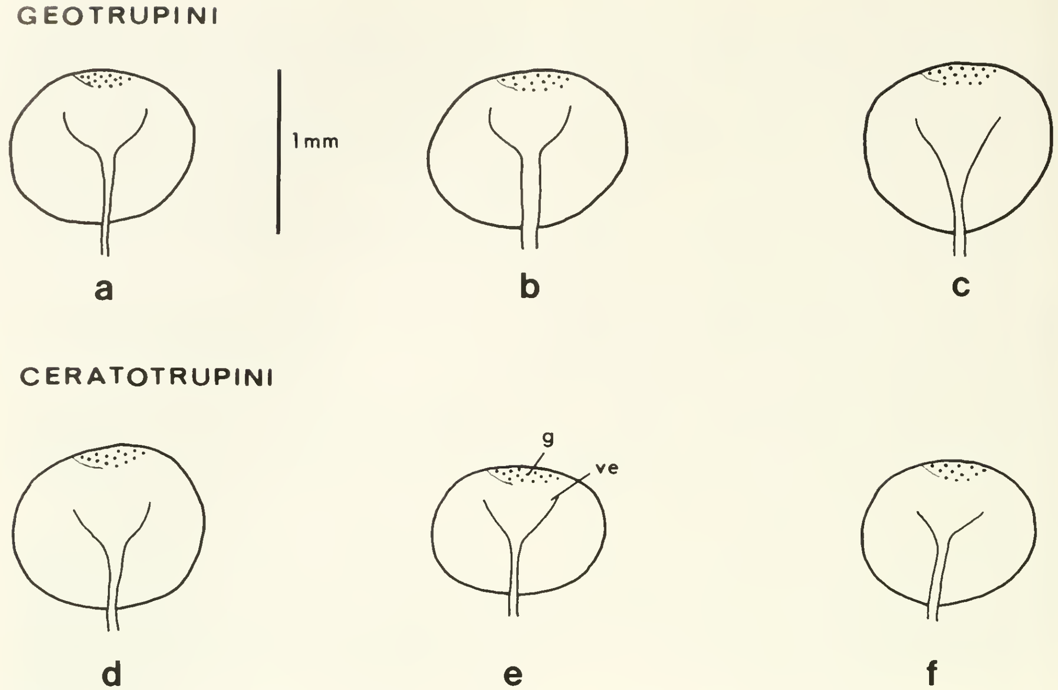

Fig. 6. Schemes of testis follicles in the two Geotrupinae tribes. Geotrupini: a, *Megatrupes cavicollis*; b, *Trypocopris vernalis*; c, *Anoplotrupes stercorosus*. Ceratotrupini: d, *Ceratotrupes bolivari*; e, *Onthotrupes nebularum*; f, *Halffterius rufoclavatus*. (g = germarium; ve = vas efferens).

Similarly, the distribution of cysts in different stages of spermatogenesis is similar in Scarabaeinae and Aphodiinae, but distinct in Geotrupinae. Nevertheless, in Scarabaeinae and Geotrupinae the germarium contains abundant sperm, while in Aphodiinae they are few. Again, cysts in different developmental stages are quite abundant in Scarabaeinae and Geotrupinae, and scarce in Aphodiinae. Another characteristic of Aphodiinae is that the primary spermatocytes are very large, a feature already described and analyzed by Virkki (1956, 1957). Largely for these reasons, Virkki (1951, 1957) considered *Aphodius* more evolved than Scarabaeinae species. Geotrupinae follicles present a structure like that of Melolonthinae and Rutelinae follicles (Virkki 1956, 1957).

Sperm bundles are distributed similarly in Scarabaeinae and Eupariini, but very differently in Aphodiini. One Aphodiini characteristic is that sperm bundles are found beneath the follicular wall, even in species that have two sizes of follicle. This type of distribution is not seen in Aphodiinae Eupariini, nor in Scarabaeinae or Geotrupinae. Virkki (1951, 1956, 1957) is the only author to have studied some Aphodiini species. However, he did not note the differences in follicle size among these species, nor the distribution of sperm bundles as described here. Eupariini have never been studied previously.

The presence of balled sperm bundles, which Virkki (1957) considers characteristic of Aphodiinae, was also observed in this study in some Scarabaeinae species: *C. telamon, G. lacordairei, D. satanas, C. moestum,* and *A. illaesum.* Balled sperm bundles were not seen in Geotrupinae.

Spermatozoa.—In Scarabaeinae species, spermatozoa measure from 90 to 600 μm in length. The longest Scarabaeinae spermatozoa (from 300 to 600 μm) are seen in Onthophagini species (except in *D. gazella,* which has spermatozoa about 90 μm long), and in Onitini Oniticellina and Dichotomiina (except in *O. mexicanus,* whose spermatozoa are about 100 μm long). The shortest spermatozoa (from 100 to 200 μm) are found in Coprina, Eurysternina, and Canthonina.

The Aphodiinae subfamily shows the greatest variation in spermatozoa length from one tribe to another. In Aphodiini, spermatozoa measure between 500 and 2,000 μm. The spermatozoa about 2,000 μm long are found in species with just one follicle size. Species with two sizes of testis follicle have spermatozoa about 1,000 μm in length in the larger follicle, and about half that length in the smaller follicle. In contrast, in Eupariini, spermatozoa are just 110 to 180 μm.

In Geotrupinae, spermatozoa length is similar in both tribes: in Geotrupini, from 126 to 137 μm, and in Ceratotrupini, from 116 to 166 μm (Fig. 4).

With regard to body length, the Aphodiini studied, except for *A. fossor,* measure from 4 to 5 mm, and are thus the smallest of the three subfamilies. Scarabaeinae and Geotrupinae measure from 7 to 15 mm in body length. Nevertheless, Aphodiini have the largest spermatozoa, if the fewest. The characteristics observed in *Aphodius*—the unusual size of spermatozoa together with the form they take of being rolled up beneath the wall of the follicle, which is spherical and small (about 22 to 50 mm)—have been described in one other species of Coleoptera, *Alagoasa bicolor* (L.) (Chrysomelidae) by Virkki and Bruck (1994). Spermatozoa of large size have also been observed in other Coleoptera, such as *Divalves bipustulatus* (F.) (Cleridae) and *Ptinella aptera* (Guerin) (Ptiliidae), species in which spermatozoa measure double the length of the body (Mazzini 1976, Taylor et al. 1982); and in some species of Diptera, such as *Drosophila littoralis* Meigen, in which spermatozoa can measure up to 19 mm (Bressac et al. 1991). In Diptera, however, the organization of cysts during spermatogenesis is different from that in Coleoptera: the tubiform testes of *Drosophila* allows the parallel elongation of sperm bundles (Tokuyasu et al. 1972), which could not occur in *Aphodius* or *Alagoasa,* which have spherical follicles.

What might the advantages be of the fewer, longer spermatozoa for Aphodiinae? According to Virkki (1973), reduction in sperm bundles reflects the total production of spermatozoa, and specialized insects tend to have fewer sperm bundles than more primitive insects. Reduction in spermatozoa number probably limits genetic variability and facilitates the adaptation of populations to specialized niches.

Male dung beetles have been studied very little. There are only few scattered data for a small number of species on the morpho-functional aspects of their reproductive apparatus. The Scarabaeinae, Aphodiinae, and Geotrupinae males lack seminal vesicles; their spermatozoa accumulate in the vas deferens, which has a muscular wall; and they have an ejaculatory duct with thick muscular walls (Pluot-Sigwalt and Martínez 1998). During mating, Scarabaeinae males form a voluminous spermatophore (Cruz and Martínez 1992). In some Aphodiinae species, spermatophores have also been observed. In *A. opisthius* spermatozoa measure about 2,000 μm, and the females have a spermatheca that measures approximately 300 μm in length (Martínez, M. I., unpublished observations). Several questions are raised by these differences between the size of the spermatozoa and that of the spermatheca in which the spermatozoa are stored following mating. How do the very large spermatozoa pass from the testes to the spermatheca? And how are the spermatozoa accomodated within the spermatheca?

With regard to nesting, Scarabaeinae species show complex nesting behavior, with some species caring for the nest until the young emerge. In this sense, Scarabaeinae appear to be the most evolved; and they have the lowest fecundity. On the other hand, Aphodiinae have the least evolved nesting behavior, and the females show the highest level of fecundity (Halffter and Edmonds 1982; Cambefort and Hanski 1991). What might the advantages be of the fewer, longer apermatozoa for Aphodiinae? According to Virkki (1973), reduction in sperm bundles reflects the total production of spermatozoa, and specialized insects tend to have fewer sperm bundles than more primitive insects. Reduction in spermatozoa number probably limits genetic variability and facilitates the adaptation of populations to specialized niches.

The questions that have been raised here define some of the next research steps about the reproductive biology of dung beetles, particularly the less well known Aphodiinae.

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